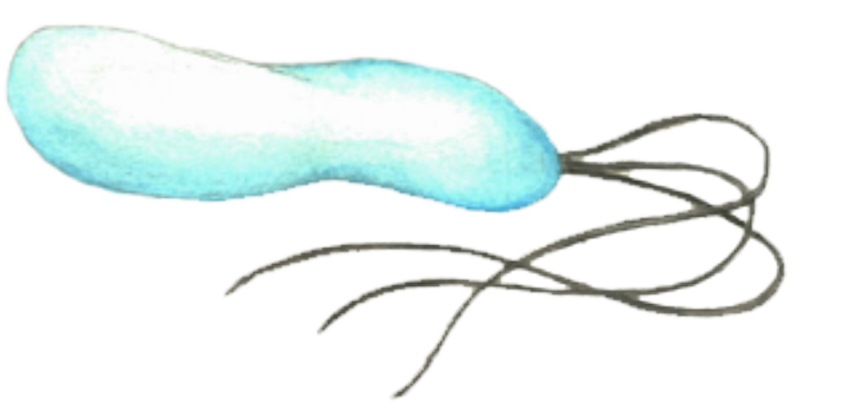
Mechanisms of host immune regulation in the *Euprymna scolopes/ Vibrio fischeri* symbiosis



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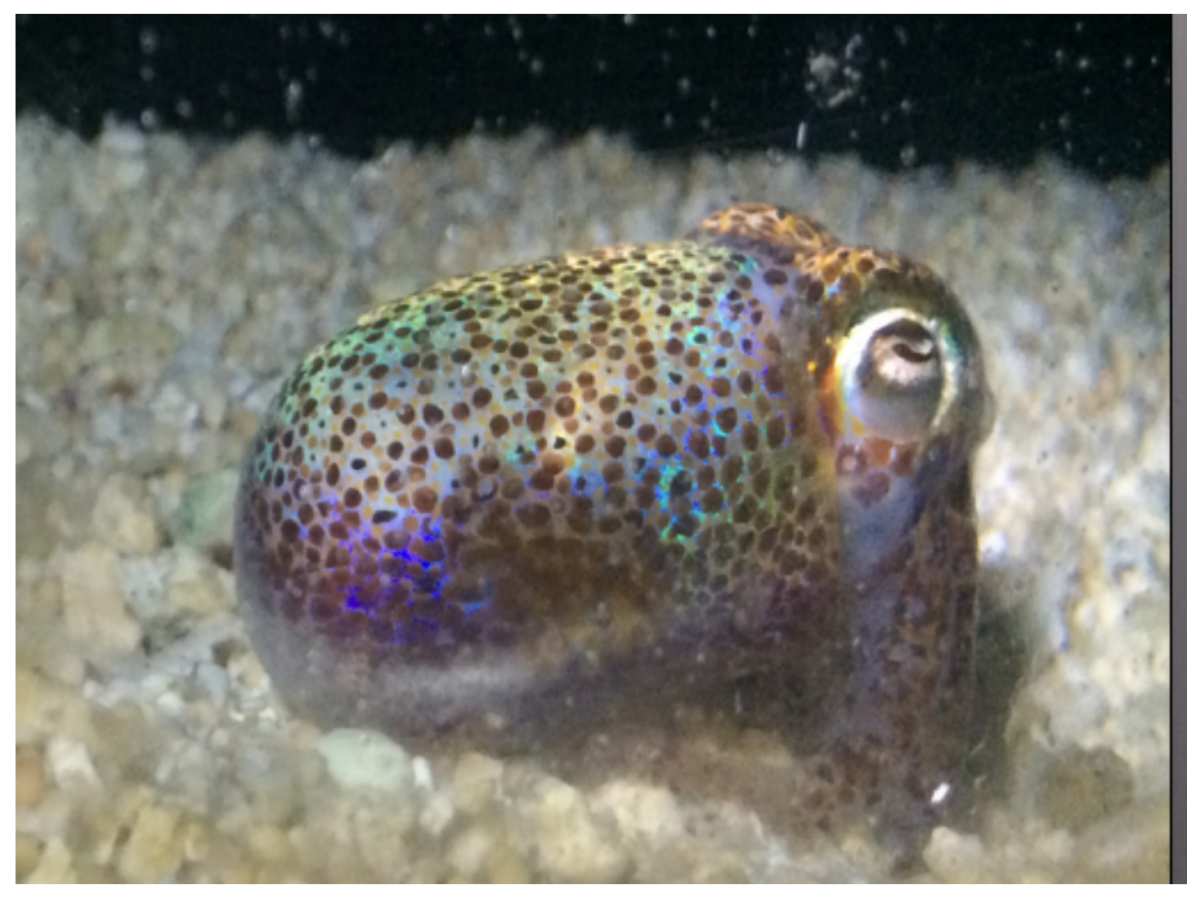
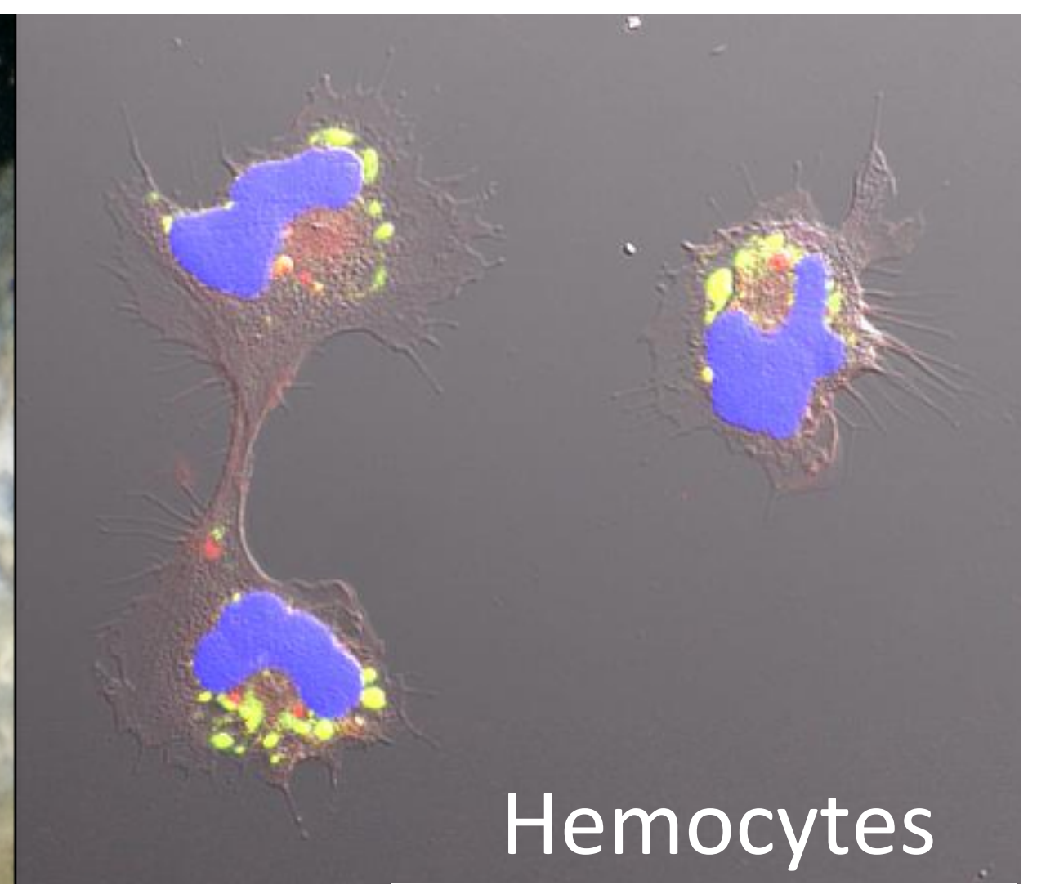
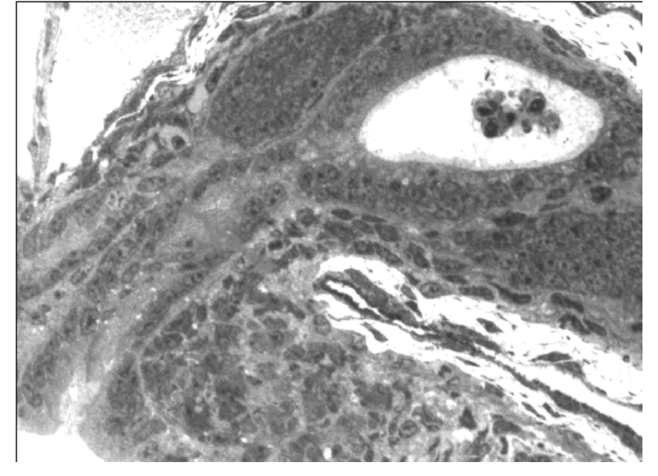
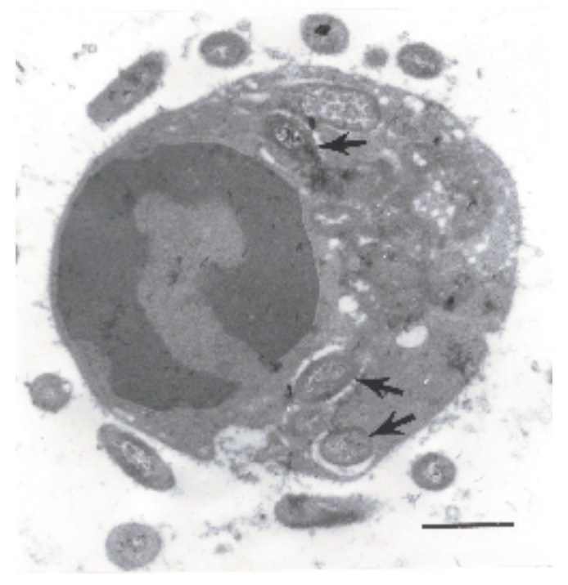
Introduction

Euprymna scolopes engages in a specific symbiosis with *Vibrio fischeri*, a bioluminescent bacterium. *V. fischeri* is housed in the light organ on the ventral side of the squid, offering counter-illumination as camouflage as the host forages at night. The cellular immune system of the Hawaiian bobtail squid consists of at least one type of blood cell with macrophage-like functions, the hemocyte.









Squid immune cells: Hemocytes

- Macrophage-like
- Easily removed for in-vitro study
- Migrate into the light organ & crypt spaces
- Have pattern recognition receptors(PRRs) that recognize microbe-associated molecular patterns (MAMPs), i.e. lipopolysaccharide and peptidoglycan

Hemocytes from adult squid from which the symbiont was removed after treatment with antibiotics bound *V. fischeri* significantly more than hemocytes isolated from colonized adults. This finding suggests that long-term colonization leads to immune tolerance of *V. fischeri*[1].

How do hemocytes distinguish between symbiotic and non-symbiotic bacteria?
Do PRR/MAMP interactions play a role in this distinction?

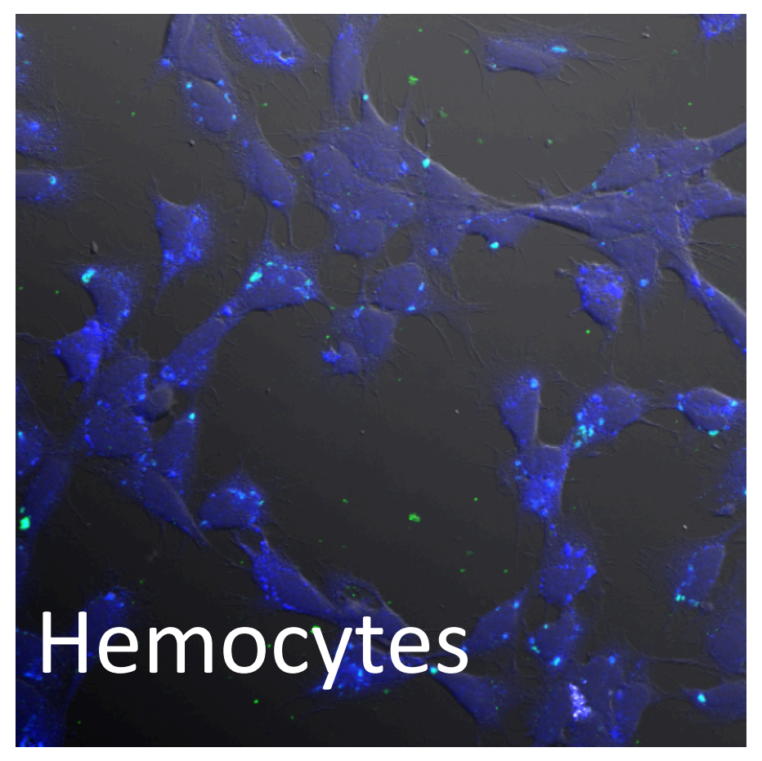
How does colonization alter hemocyte binding to *V. fischeri*?

Does *E. scolopes* have multiple hemocyte types?

- Most mollusks have 2 morphologically distinct cell types[2]
- Coleoid cephalopod hemocyte morphology varies greatly
  - Octopus 2 or 3 subtypes[3,4]
  - Closely related *Sepia officinalis* has 1 morphological type[5]
- Anecdotal evidence suggests multiple hemocyte phenotypes

Hypothesis: There are multiple hemocyte subtypes in *E. scolopes*

Experiment: *Euprymna* hemocyte morphology was investigated using ICC and flow cytometry



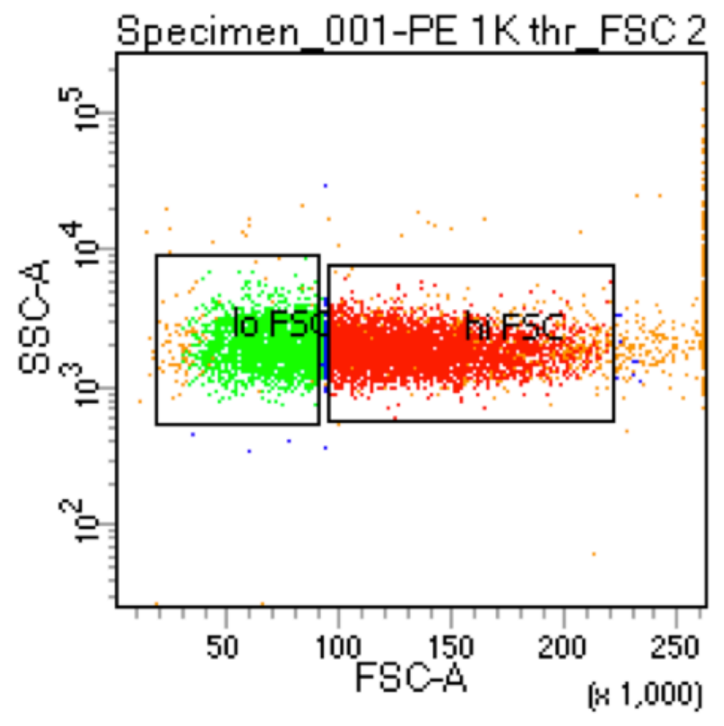


Figure 1 forward and side scatter of adult hemocytes isolating from hemolymph

Hemocyte Differentiation

Conclusions

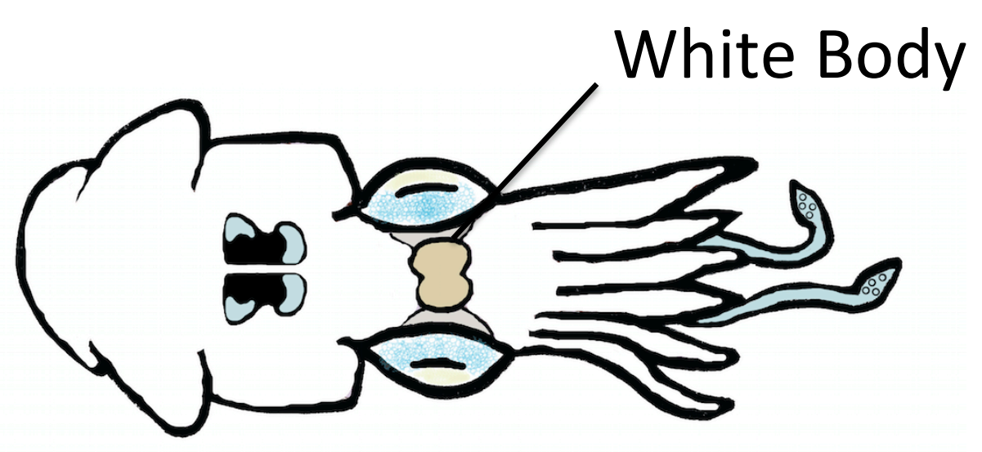
- Only one morphological cell type observed, consistent with previous observations[1,6]

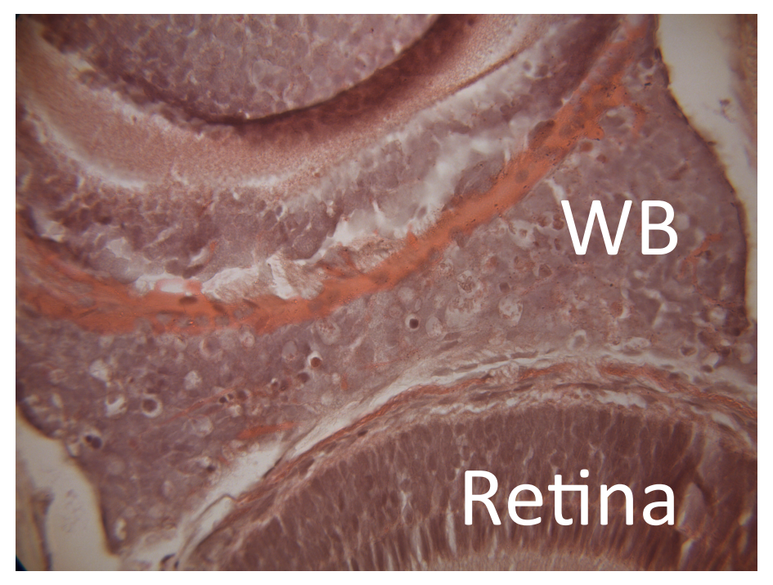
Morphologically identical cells may serve different functions

Future Directions

- Use flow cytometry to determine proportion of hemocyte population expressing known hemocyte proteins
  - Peptidoglycan Recognition Protein 3, 4, and 5
  - Cathepsin L2
  - Galectin 1&2
  - Toll-like Receptor
  - CD109
  - Complement component C3
- Characterize putative populations based on protein profiles, tissue type, and gene expression

Does colonization affect hematopoiesis?





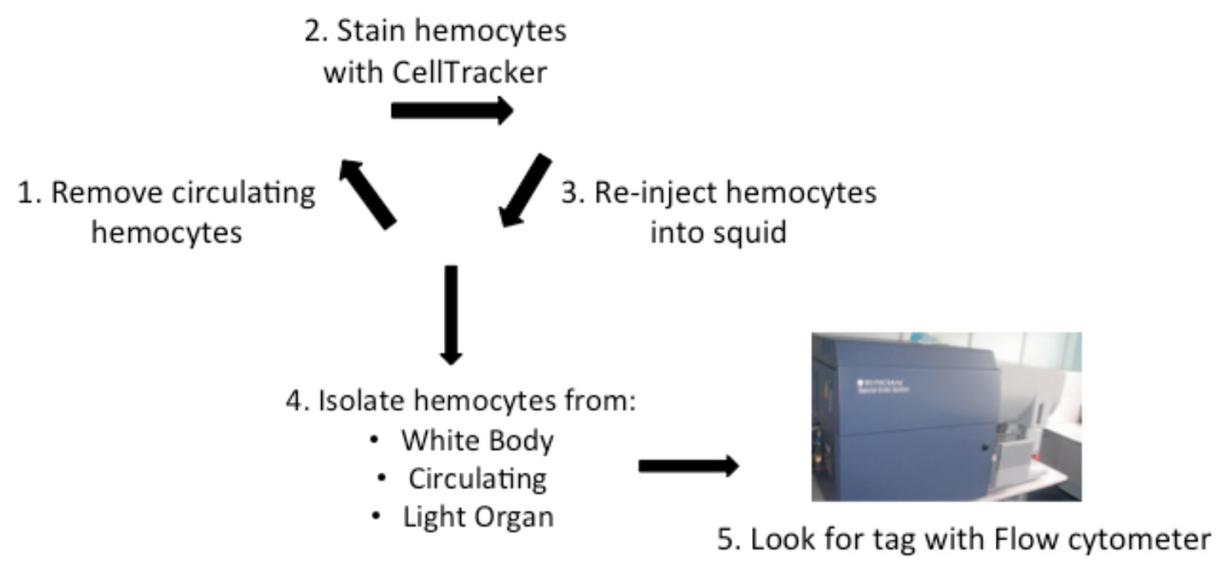
White body

- Site of hematopoiesis in coleoid cephalopods
- Multi-lobed organ between eye and optic lobe
  - Highly vascularized
- Contains cells with high mitotic activity

Hypothesis: Colonization of the light organ by *V. fischeri* influences hematopoiesis

In order to know whether hematopoiesis changes in response to colonization, the process needs to be characterized. It is unknown whether hemocytes in the light organ traffic back to the white body.

Experiment: Do circulating hemocytes traffic back to white body?



1. Remove circulating hemocytes
2. Stain hemocytes with CellTracker
3. Re-inject hemocytes into squid
4. Isolate hemocytes from:
   - White Body
   - Circulating
   - Light Organ
5. Look for tag with Flow cytometer

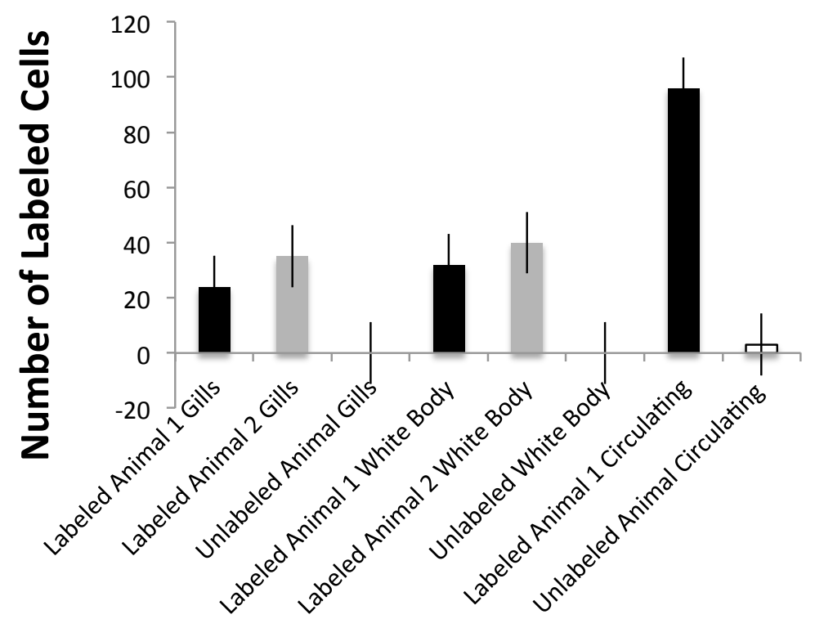


Figure 2 Cell tracker positive cells found in white bodies, gills, and and circulation of 2 animals injected with CellTracker labeled cells, and one CellTracker unlabeled animal

Conclusions:

- Circulating hemocytes *may* traffic back to white body

Future Directions

- Determine whether light organ hemocytes traffic back to white body by performing live tracing experiments using flow cytometry and sectioning
- Electron microscopy of white body to characterize morphology & hemocyte developmental stages

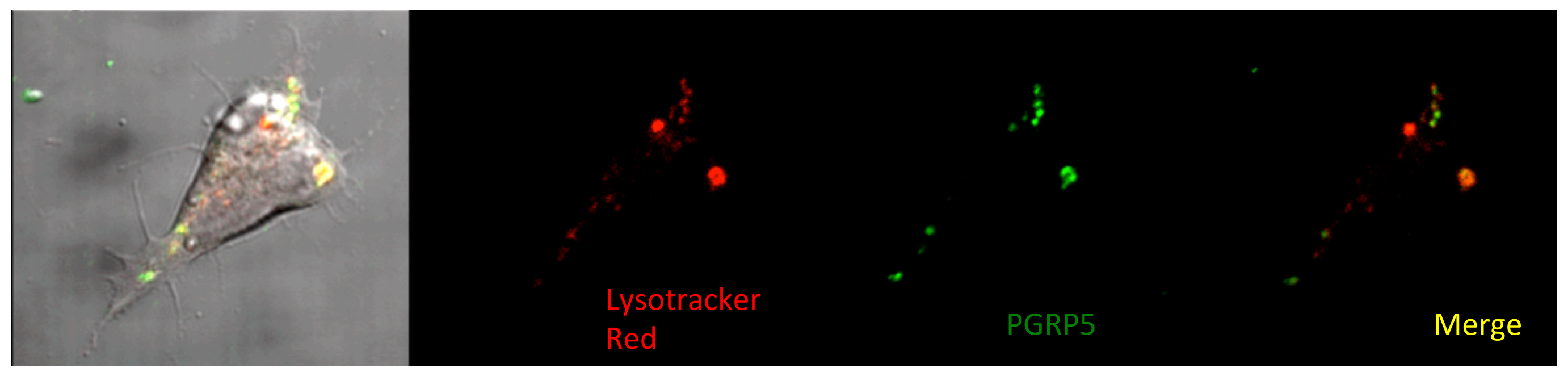
What is PGRP5's role in symbiosis?

PGRP5

- Peptidoglycan recognition receptor
- One of the most highly expressed transcripts in hemocytes[7]
- Colonization leads to a significant decrease in protein abundance of PGRP5 in hemocytes[8]
- Predicted to have amidase activity[7]
- Similar to *Drosophila* PRGP-SC, a secreted PGRP
  - PRGP-SC2 promotes *Drosophila* gut homeostasis[9]
- Other EsPGRP's have been shown to play a role in symbiosis [10, 11]

Hypothesis*:* PGRP5 is located in lysosomes and is secreted directly or via lysosomal exocytosis

Experiment 1: Does PGRP5 co-localize with lysosomes?



Conclusions:

- PGRP5 is localized to vacuoles (A. Collins), often lysosomes

Future directions

- Determine whether PGRP5 has a signal peptide
- Confirm that PGRP5 is secreted
- Express a recombinant protein in *E. coli* or insect cells
- Confirm amidase activity in vitro with peptidoglycan and its derivative, tracheal cytotoxin
- Determine how *V. fischeri* modulates PGRP5 expression

Summary

- *E. scolopes* has one morphological immune cell type, but molecular subtypes have yet to be determined.
- Circulating hemocytes may traffic to the white body, but whether they deliver a "colonization-signal" to progenitor cells remains to be determined.
- PGRP5 often co-localizes with lysosomes in hemocytes. Whether PGRP5 interacts with phagocytized bacteria or is secreted (solely or via lysosomal exocytosis) remains to be determined.

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